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THE ACUTE TOXICITY OF ISOPROPYLAMINE AND 2-METHYLCYCLOHEXANOL

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The toxicity of isopropylamine (IPA) and 2-methylcyclohexanol (2-MCH) was determined. Acute 48-hr bioassays were performed using the water flea, Daphnia magna. Ninty-six hour, growth inhibition assays were conducted using freshwater green eucaryotic algae, Selenastrum capricornutum, and Ankistrodesmus falcatus. Isopropylamine was more toxic to the test organisms than 2-MCH. The 48-hr EC50 of IPA to daphnia was 91.5 mg/L. When buffered with 10% HCl, the 48-hr EC50 of IPA increased to 148.8 mg/L. The 96-hr IC50 of buffered IPA to S. capricornutum was 118.4 mg/L. The 48-hr EC50 of 2-MCH to daphnia was 267.0 mg/L. The 96-hr IC50 of 2-MCH to algae was 395.0 mg/L.

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PREFACE

The work described in this report was authorized under Project No. 1C162622A554, Chemical Munitions. This work was started in April 1988 and completed in October 1988.

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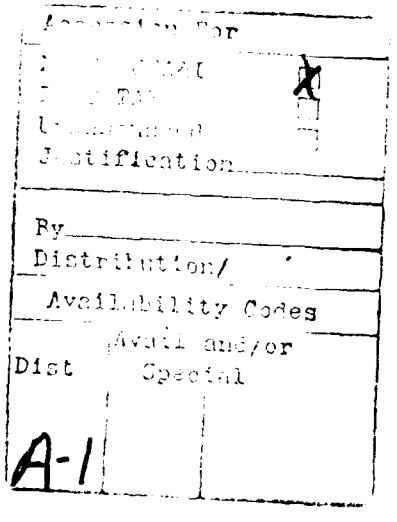

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THE ACUTE TOXICITY OF ISOPROPYLAMINE AND 2-METHYLCYCLOHEXANOL

1. INTRODUCTION

To provide adequate protection to the environment, a data base must be established for all chemicals. A data base provides toxicity information that can be used in estimating environmental impact. Some of the first steps in establishing the aquatic toxicity of a compound are the daphnia and algae bioassays. These bioassays are short term, widely used, and have well-documented test procedures.

In this study, the acute toxicity of isopropylamine (IPA) and 2-methylcyclohexanol (2-MCH) is examined. Acute 48-hr bioassays were performed using the water flea, Daphnia magna, and 96-hr, growth inhibition assays were conducted using the freshwater green eucaryotic algae, Selenastrum capricornutum (S. capricornutum) and Ankistrodesmus falcatus (A. falcatus).

Table 1 shows some of the physical and chemical properties of the test compounds. Isopropylamine is used in the synthesis of pharmaceuticals, pesticides, and dyes.[1] 2-Methylcyclohexanol is used in lacquers, films, various solvents, and soaps.[2]

2. METHODS AND MATERIALS

Isopropylamine (lot #1295-23) was obtained from Dr. Eugene Olajos, Research Directorate, U.S. Army Chemical Research, Development and Engineering Center (CRDEC). Isopropylamine caused the pH value to shift when added to the media. Therefore, duplicate tests were run using buffered (pH 7.2, using 10% HCl) IPA solutions.

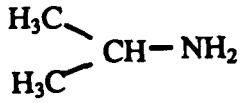
2-Methylcyclohexanol was obtained from Mr. Charles Crouse, also of the Research Directorate, CRDEC. All tests conformed to current U.S. Environmental Protection Agency (EPA)[3] and American Society for Testing and Material (ASTM)[4] guidelines.

2.1 Daphnia Assays.

D. magna were obtained from Dr. Freda Taub at the University of Washington (Seattle, WA). Daphnia were reared in the laboratory as described by Goulden et al.[5] Daphnid stock cultures were fed a mixture of A. falcatus, S. capricornutum, and Chlamydomonas reinhardi 90. The culture media was prepared from municipal drinking water that was hardened to 132 ppm total $CaCO_3$.[4] The pH was adjusted to 7.2-7.5. Ten neonates less than 24 hr old were placed into 250-mL glass beakers filled with 100 mL of the test solution. The test beakers were placed into an incubator with a light-dark cycle of 16:8 hr with 315 ft candles of light at 20 °C. Two replicates were used in each test. Daphnia were gently touched with a pastier pipet at 24 and 48 hr. If the daphnia could not swim actively for 15 s, immobilization (mortality) was recorded. The effective concentrations at which 50% of the organisms are immobilized (EC50) were computed using the probit analysis prepared by Stephan.[6]

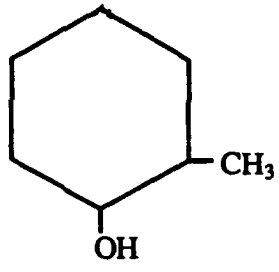
Table 1. Some Physical and Chemical Properties of the Test Compounds[1,2]

Isopropylamine



Synonyms:	2-aminopropane, monoisopropylamine, 2-propanamine
Description:	colorless liquid, ammonia order, basic
Mol wt:	59.11
Boiling pt:	33-34 °C
Density:	0.694
Flash pt:	-26 °C
Solubility:	miscible with water
Toxicity:	oral rat LD50 = 0.82 g/kg

2-Methylcyclohexanol



Synonyms:	O-methylcyclohexanol, 2-methylcyclohexyl, alcohol, hexahydrocresol and hexahydromethylphenol
Desription:	colorless liquid or pale yellow, menthol odor
Mol wt:	114.19
Boiling pt:	173.0-175.3 °C
Freezing pt:	-50 °C
Density:	0.913
Flash pt:	67.7 °C
Solubility:	3-4% in water
Toxicity:	oral rat LD50 = 2.0 g/kg

The percent mortality values were plotted against concentration. A least-square regression line was drawn, and the EC50 was tabulated graphically. The graphically determined EC50 was used in verifying the probit analysis.

2.2 Algal Growth Inhibition Assays.

S. capricornutum and A. falcatus were also obtained from Dr. Freda Taub, University of Washington (Seattle, WA). Stock cultures of algae were maintained on 1.5% Difco-Bacto agar slants. Test algae were grown in a semiflow-through culture apparatus on T82MV(7) and taken during log phase growth for inoculation into the test flasks. Five-hundred milliliter Erlenmeyer flasks with ground glass stoppers were used as test chambers. One-hundred milliliters of media was placed in each test chamber and inoculated with approximately 4.0×10^4 algal cells per milliliter. The algae were placed in an incubator under the conditions described above for the daphnia 48-hr assays. Using a Newbauer Counting Chamber, cell densities were determined every 24 hr for 5 consecutive days. The area under the growth curve (A) was calculated using the following equation:

$$A = \frac{(N_0 + N_1) - 2N_0}{2} \times (t_1) + \frac{(N_1 + N_2) - 2N_0}{2} \times (t_2 - t_1)$$

$$+ \frac{(N_n - 1 + N_n) - 2N_0}{2} \times (t_n - t_n - 1) \qquad (1)$$

where

N_0 = number of cells at t_0

N_1 = number of cells at t_1

N_n = number of cells at t_n

t_0 = time zero

t_1 = time of first measurement

t_n = time of the n^{th} measurement

The percent inhibition was calculated using the area under the growth curve. The following equation was used to calculate the percent inhibition (%In):

$$\%In = \frac{A_c - A_t}{A_c} \times 100 \qquad (2)$$

where A_c = area of control growth curve, and A_t = area of treatment growth curve.

The percent inhibition values were plotted against the concentration. A least-square regression line was drawn, and the concentration at which algal growth is reduced to 50% of the control (IC50) was determined. Analysis of Variance was run on the replicates to determine if any of the groups were significantly different. Dunnett's test was conducted to determine which treatment groups were different from the control.

3. RESULTS

Isopropylamine caused extreme pH-value shifts when added to the media (Figure 1); the dissolved oxygen remained unchanged. The unbuffered 48-hr EC50 of IPA to daphnia was 91.5 mg/L. The buffered 48-hr EC50 of IPA to daphnia was 148.8 mg/L.

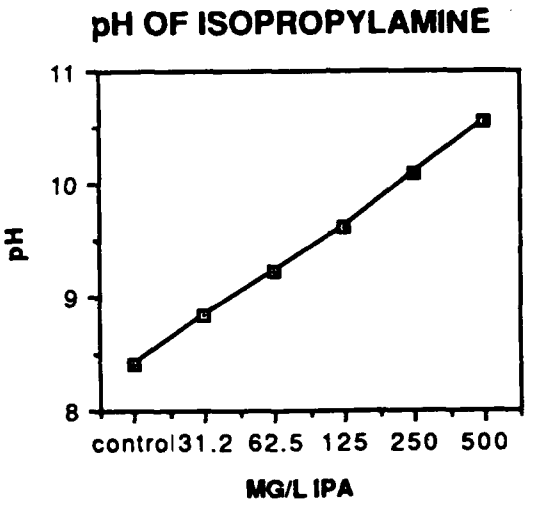


Figure 1. pH Encountered During the Unbuffered Assays.

Unbuffered solutions of IPA caused a 100% decrease in algal growth (S. capricornutum) at concentrations down to 62.5 mg/L. When buffered, the 96-hr IC50 of IPA to S. capricornutum was 118.4 mg/L. Figure 2 shows algal growth curves subjected to buffered and nonbuffered solutions of IPA. Algal growth, in the buffered and nonbuffered IPA tests, was significantly different from the control at $p \leq 0.05$.

2-Methylcyclohexanol was the less toxic of the two test compounds. The pH value and dissolved oxygen remained unchanged. The 48-hr daphnia EC50 = 267.0 mg/L. The 96-hr IC50 of 2-MCH to A. falcatus was 395.0 mg/L. At 100 mg/L of 2-MCH, algal growth was unchanged. At 250, 500, 750, and 900 mg/L of 2-MCH, algal growth was significantly reduced at $p \leq 0.05$.

4. DISCUSSION

Isopropylamine was more toxic than 2-MCH. The amine group in IPA caused a pH value shift when added to the media. Buffering the IPA solutions reduced the EC50 and IC50 from 38 to 47%, depending on test organism. However,

the IPA solutions were still ranked 4 (on a scale of 0-9 with 9 being the most toxic) on the scoring criteria for aquatic toxicity.[8] If released into an aquatic ecosystem, the impact of IPA would depend on the buffering capacity of that system.

2-Methylcyclohexanol was ranked 2 on the scoring criteria for aquatic toxicity and exhibited no adverse effects on pH or dissolved oxygen.

5. CONCLUSIONS

Isopropylamine is more toxic than 2-MCH with a scoring criteria for aquatic toxicity of 4 and 2, respectively. Buffering solutions of IPA showed toxicity reduction. The buffering capacity of the ecosystem will play a strong roll in reducing the toxicity of IPA if an accidental spill occurs. A safety factor of 20 is commonly used when trying to estimate a not-to-exceed limit to prevent chronic effects. Using the most sensitive organism tested, the not-to-exceed level for buffered IPA is 5.9 mg/L. The not-to-exceed level for 2-MCH is 13.3 mg/L.

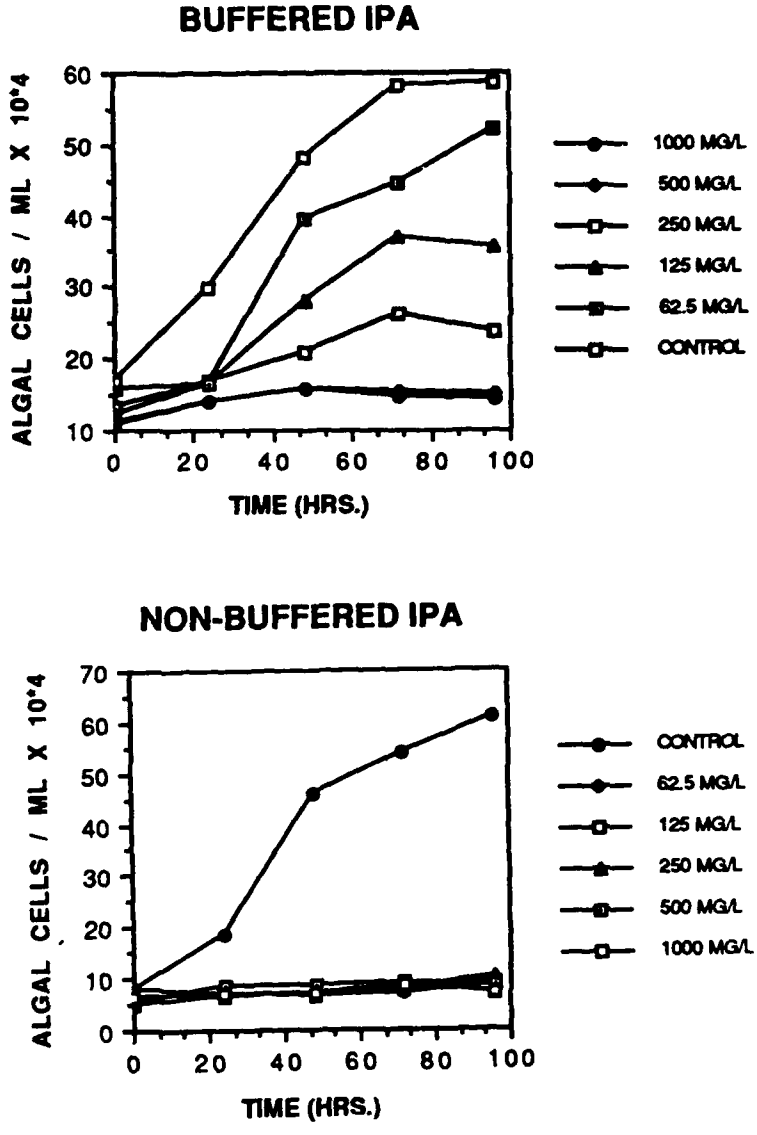


Figure 2. Growth Curves of S. capricornutum Exposed to Buffered and Nonbuffered IPA

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